REMARKS

Support in the specification for the amended and new claims is shown in the following table:

LANGUAGE/CLAIM	SPECIFICATION SUPPORT
Claim 1.	(Grammatical error, suggestion by the Examiner.)
Claim 5.	Page 12, lines 24 and 25.
Claim 8.	Original claim 1.
Claim 10.	Page 6, lines 1-4.
Claim 12.	Page 6, lines 1-4.
Claim 13.	U.S. Pat. No. 6,267,959, incorporated by reference (at page 24, line 20 and 51, lines 18 and 19).
Claim 14.	Typographic error (correct antecedent).
Claim 15.	Typographic error (correct antecedent) and page 6, lines 11 and 12.
Claim 16.	Typographic error (correct antecedent).
Claim 17.	Page 12, lines 24 and 25 (electrophoresis is a type of gel filtration [i.e., type of chromatography] and as such it is also known for fractionating proteins on the basis of mass).
Claim 18.	Page 6, lines 2-4.
Claim 19.	Typographic error (correct antecedent).

LANGUAGE/CLAIM	SPECIFICATION SUPPORT
Claim 25.	Page 32, lines 11-25.
Claim 26.	Page 42, lines 15-22.
Claim 27.	Page 42, lines 18 and 19.
Claim 28.	Page 42, lines 15-22.
Claim 29.	Page 43, lines 2-9.
Claim 30.	Page 1, lines 19 and 20.
Claim 31.	Page 42, lines 15-22.
Claim 32.	Original claim 1.
Claim 33.	Original claim 4.
Claim 34	Page 1, lines 19 and 20.
Claim 35.	Page 1, lines 19 and 20; page 14, lines 10-20.
Claim 36.	Page 30, line 23 to page 31, line 9.
Claim 37.	Page 30, line 23 to page 31, line 9.

Accordingly, no new matter has been added and entry of the amendments and new claims is requested respectfully.

I. Summary of the Office Action

The specification, the Declaration and the drawings are objected to on formal matters. Claims 1-24 are rejected as being either anticipated by or obvious over the acted art. Each of the rejections are addressed below.

II. Summary of the Response

The specification has been amended to reflect the changes in the designation of the drawings and to comply with the requests/suggestions of the Examiner.

Claim 2 has been cancelled. Claims 1, 5, 8, 10, 12 and 14-19 have been amended and claims 25-38 have been added to further describe the present invention. The Applicants traverse the outstanding rejections against claims 1-24 as amended-in-part.

III. Listing of References

The Examiner asserts that the list of references at the end of the specification does not comply with 37 C.F.R. §1.98(b) and as such, they have not been considered.

The references listed at the end of the specification are freely available to those skilled in the art. These references are offered to provide a non-limitative collection of source materials concerning general art recognized information, concepts and methods that may be of interest to the skilled artisan in those fields embraced by the instant inventive concept. Further, they serve, in part, to buttress the facts as presented in the background of the specification.

IV. Oath/Declaration

The Examiner asserts that the Oath/Declaration does not identify the mailing or post office address of each inventor. The Information Data Sheet filed with this application correctly identifies each coinventor and mailing address including the zip code. Appended hereto is a copy of the Information Data Sheet as originally filed.

V. Objections

Drawings

The Examiner asserts that the drawings are objected to because Figures 1A-AD [sic] should be amended to include all vertical and horizontal units. Further, Figure 3 is suggested to be modified to show molecular weight units.

Accordingly, Applicants have modified the drawings to include the changes as recommended by the Examiner in the Action. Further, no prohibited new subject matter has been added. Modified drawing figures are appended hereto.

Respectfully, Applicants request that the changes be accepted and the corrected drawings entered as informal.

Specification

The specification stands objected to for allegedly failing to provide proper antecedent basis for the claimed subject matter.

Accordingly, Applicants have amended the specification to reflect the elements recited in claim 13 and claims 10 and 11 as suggested by the Examiner. Further, no new subject matter has been added.

Respectfully, Applicants request that the amendments to the specification be entered. In view of these amendments, Applicants submit that the objections with respect to the specification are overcome.

Claims

Claims 1, 14, and 16 stand objected to for alleged informalities.

While not acquiescing to the reasoning offered by the Examiner, in order to expedite prosecution of the application, Applicants have amended the claims to more clearly define the invention.

Respectfully, Applicants request that the amendments to the claims be entered. In view of these amendments, Applicants request that the objection with respect to the claims be withdrawn.

VI. Rejections Under 35 U.S.C. §112, Second Paragraph

Claims 1-20 stand rejected under 35 U.S.C. §112, second paragraph for allegedly being indefinite. Regarding claims 2, 20 and 21, as these claims has been canceled, the rejection is moot with respect to said claims. Applicants, respectfully traverse the rejections as applied against claims 1, 3-24 as amended-in-part.

The Examiner asserts that claim 1 is indefinite because it recites "above about."

However, the term listed is not *per se* indefinite. A brief keyword search for U.S. Patents limited to claims (1976-present) would show that 6608 issued U.S. patents recite the term "above about" in the claims. Thus, "above about" is a well-recognized term of art. An artisan would well recognize the metes and bounds of the term and of claims containing the term.

The Examiner alleges that the recitation "the filtration limits of a normal kidney" has insufficient antecedent basis. However, the term is being taken out of context in the Action and is in fact being used by Applicants in the claim to define a range of molecular weights of proteins embraced by the claim. Further, the filtration limits of a normal kidney is well known (e.g., search limited to the specification for filtration limits of the normal kidney in the patent database would result in 11 hits). As such, the characterization of the phrase as "lacking sufficient antecedent basis" is inappropriate, since in reading the phrase in context one of skill in the art would understand the metes and bounds of the range described.

Claim 5 is alleged to lack antecedent basis in view of the phrase "said concentrating step." While not acquiescing to the reasoning offered by the Examiner, in order to expedite prosecution of the application, Applicants have amended claim 5.

Claim 10 is alleged to lack antecedent basis in view of the phrase "the elution." While not acquiescing to the reasoning offered by the Examiner, in order to expedite prosecution of the application, Applicants have amended claim 10.

Claim 15 is alleged to lack antecedent basis and is vague and indefinite because of the antibody comprising the column. While not acquiescing to the reasoning offered by the

Examiner, in order to expedite prosecution of the application, Applicants have amended claim 15.

Claim 17 is alleged to be vague and indefinite with respect to the term "said separating step." While not acquiescing to the reasoning offered by the Examiner, in order to expedite prosecution of the application, Applicants have amended claim 17.

Claim 19 is alleged to lack antecedent basis in view of the phrase "said deflecting step." While not acquiescing to the reasoning offered by the Examiner, in order to expedite prosecution of the application, Applicants have amended claim 19.

Claims 20 is alleged to be vague and indefinite, asserting that there "is no step (c) recited in claim 1." In view of the amendments to claim 1, the rejection should be withdrawn as moot.

Claim 20 is also alleged to be vague and indefinite because "data is not always fixed and the image can be changed." Further, the Examiner suggests that because the data is variable, the image is not consistent and concludes that the claim is vague. However, Applicants point out that while the image is dependent on the sample (where the sample may vary), for any given sample the image is consistent. As such, the skilled artisan would understand the metes and bounds of the claim. Therefore, the claim is not vague.

Further, claim 20 is alleged to be vague and indefinite because the Examiner suggests that it is unclear how an image provides linkage to an annotation. As is well known in the art, a computer image may be made to contain one or more invisible regions called "hotspots," which can be assigned to links (i.e., a Windows command that takes several

programs and subprograms that were meant to be used together, but were written separately, and combines them into one). Typically, an image can make available stored information (e.g., annotations: notes stored on your local hard disk which are available each time you access an image) using visual cues (e.g., spots on the image of a 2D gel). By clicking on said cues, which can be located at different places on the image, the stored information is accessed. Thus, for example, an image of a 2D gel can be assigned hotspots on various parts of said image, each hotspot having a link to the hard drive, whereby when a visual cue is clicked, a particular annotation (e.g., patient information) is made available (e.g., "pops-up" on the screen).

As all of this information is well known in the art, the skilled artisan would know the metes and bounds of the claim. Therefore, the claim in question is not indefinite.

Claim 21 is alleged to be indefinite because it is suggested that an adapted image display means is unclear. Simply put, it is well know that to "adapt" in computer or electronics arts means to make a device (e.g., an image displaying means) compatible with the system(s) to which it is attached (e.g., an image data storage means). Applicants suggest no alternative meaning. As such, one of skill in the art would know the metes and bounds of the claim. Therefore the claim is not indefinite.

Accordingly, for these reasons, Applicants respectfully request that the rejection be withdrawn against claims 1, 3-24 as amended-in-part.

VII. Rejections Under 35 U.S.C. §102(b)

A. Claims 1 and 3-5 stand rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Liu et al (U.S. Pat. No. 5,492,834).

The Examiner asserts that Liu et al. teach a method of detecting proteins in urine samples by applying a sample to a size exclusion gel, wherein the cutoff is at least 6,000. Further, such a gel is alleged to serve to fractionate proteins, leading to analysis of such fractions where the molecular weights of the proteins are greater than the exclusion molecular weight of the gel.

However, examination of the reference demonstrates that Liu et al. do not disclose a method comprising "separating a fraction having proteins or peptides with a molecular weight above about 3 kDa and below the filtration limits of a normal kidney," nor recovery of proteins from this separated fraction (see instant amended claim 1). Further, as can be seen in amended claim 1, there is an express upper limit element, namely below the filtration units of the kidney. Such an upper limit is not taught in the Liu et al. reference. As such, the resulting fraction isolated/analyzed isolated by the method of Liu et al. (e.g., comprising high molecular weight proteins such as albumin and the like, which are normally above the filtration limit of a normal kidney) would not be the same using the method steps of the instant claims.

The low molecular weight proteins (or fragments of larger proteins) obtained by the instant method as claimed may be made anywhere in the body and represent breakdown products and potential disease markers. For example, Figure 2A of the instant invention

demonstrates that serum contains almost no proteins below about 30,000 Da (30 kDa). These low molecular weight proteins are rapidly removed and appear in the fraction as envisaged by the claims (see e.g., Figure 2B). As such, disease induced expression of low molecular weigh proteins, which may be present in low abundance in the blood, will appear in higher quantities in the urine fraction resulting from the method as recited claimed invention. Liu et al. passes urine through a size exclusion column and recovers all proteins above 6,000 Da (i.e., 6 kDa). There is no separation of the low molecular weight proteins recovered by the instant method from high molecular weight proteins (i.e., those above the filtration limits of a normal kidney).

Moreover, Liu et al. specifically exclude low molecular weight proteins (i.e., materials sought to be analyzed by the instant claimed method) as indicated in column 1, lines 11-16, of Liu et al.:

> "More particularly, the present invention involves methods for **removing low molecular weight** compounds from urine samples . . .". (Emphasis added).

Further, in contrast to the position of the Examiner regarding fractionation, at column 6, lines 39, of Liu et al.:

> "Similarly, the pore size is large enough to trap, **WITHOUT FRACTIONATING**, low molecular weight sample constituents which interfere with the analysis of the analytes of interest." (Emphasis added).

The present invention as claimed expressly resolves low molecular weight proteins for recovery and analysis. In contrast, low molecular weight proteins are seen as a hindrance of the further analysis envisaged by Liu et al. For example, at column 6, lines 50-54 of Liu et al.:

> "Alternatively, size exclusion gels . . . fractionate the sample components so that the analytes of interest elute from the gel and the interfering constituents remain in the gel."

The analytes of interest in Liu et al. are the low molecular weigh proteins referred to in column 1, lines 11-16 and column 2, lines 32-34. As stated in *Hybritech Inc. v. Monoclonal Antibody, Inc.* 802 f.2d 1367, 231 USPQ 81(Fed. Cir. 1986), "It is axiomatic that for prior art to anticipate under 102 it has to meet every element of the claimed invention." Further, "anticipation requires identity of the claimed process of the prior art; the claim process, including each step thereof, must have been described or embodied, either expressly or inherently, in a single reference." *Glaver Bell Societe Anonyme v. North Lake Marketing and Supply Inc.* 45 F.3d 1550 33 USPQ 2d 1496 (Fed. Cir. 1995). In other words, as stated in MPEP § 706.02, for anticipation under 35 U.S.C. §102, the reference must teach every aspect of the claimed invention either explicitly or inherently.

Since the reference cited does not teach separating a fraction having proteins or peptides with a molecular weight above about 3 kDa and below the filtration limits of a normal kidney (i.e., a step reciting an express upper limit for the fractionating of low

molecular weight proteins), Liu et al. does not meet every limitation of the claimed invention. Therefore, Liu et al. does not anticipate amended claims 1 and 3-5. Liu et al. also does not read on new claims 25-38 since these claims are dependent from claim 1.

Respectfully, in view of the arguments presented above, Applicants submit that new claims 25-38 are allowable.

B. Claims 1, 3, 4, 8, 9 and 17 stand rejected under 35 U.S.C. §102(b) as allegedly being anticipated by Myrick et al (Applied and Theoretical Electrophoresis (1993) 3:137-146). Applicants respectfully traverse the rejection as it stands against amended claims 1, and 3, 8, 9 and 17 (amended in part). The Examiner asserts that Myrick et al. teach a method of determining a protein in a urine sample using centrifugation for concentrating samples and further subjecting said samples to micro-concentration via a 10KDa cutoff membrane.

However, examination of the reference demonstrates that Myrick et al. do not disclose a method comprising "separating a fraction having proteins or peptides with a molecular weight above about 3 kDa and below the filtration limits of a normal kidney," nor recovery of proteins from this separated fraction (see instant amended claim 1). Further, as can be seen in amended claim 1, there is an express upper limit element. Such an upper limit is not taught in the Myrick et al. reference. Thus, the fraction isolated in Myrick et al. is contaminated with components that would not be found using the method steps of the instant claims. For example, isolating high molecular weight proteins, such as albumin, would be relatively certain using the method of Myrick et al. as it is designed for examining kidney damage from

cadmium exposure (i.e., loss of kidney function is correlated with increased abundance of high molecular weight proteins as can be seen by recovery of proteins as high as 130KDa).

Further, examination of the reference demonstrates that the disclosure is directed 1) removal of low molecular weight proteins without analysis and 2) use of different steps, i.e., the method as disclosed is not the same as the claimed invention. For example at page 139, column 1, paragraph 1:

> "**TO REMOVE LOW MOLECULAR WEIGHT ANALYTES, . . . POLYPEPTIDES** . . . and to concentrate urinary proteins with M_r > 10,000 Da, . . . each urine sample was ultrafiltered with a 10,000-Da cutoff membrane . . .." (Emphasis added).

Thus, in contrast to the suggestion by the Examiner regarding recovering an anticipatory retentate, Myrick et al. expressly discard fractions comprising the lower separation limit as expressly recited in the instant claims.

Further, the method steps as claimed require fractionation, separation, recovery of low molecular weight proteins and analysis of low molecular weight proteins. In Myrick et al. the steps are centrifugation, centrifugation to remove [discard] lower molecular weight polypeptides, denaturation, ultrafiltration and analysis of the cleared, denatured retentate (at page 139, column 1, paragraph 1, *"Sample Processing"*). These steps do not include retention/resolution of low molecular weight proteins and analysis of said proteins. The present invention as claimed recites fractionating low molecular weight proteins for recovery and analysis.

Further, the samples of Myrick et al. are denatured and subsequently ultrafiltered (see page 139, column 1, lines 17-21). The denaturation will breakup many proteins and protein complexes, which will be lost during ultrafiltration as the molecular weight cut-off is 10 KDa.

Moreover, Myrick et al. are looking for kidney damage by surveying kidney proteins. By contrast, the instant claims look for proteins, such as plasma proteins, which are found in the urine.

As stated in *Hybritech Inc. v. Monoclonal Antibody, Inc.* 802 f.2d 1367, 231 USPQ 81(Fed. Cir. 1986), "It is axiomatic that for prior art to anticipate under 102 it has to meet every element of the claimed invention." Further, "anticipation requires identity of the claimed process of the prior art; the claim process, including each step thereof, must have been described or embodied, either expressly or inherently, in a single reference." *Glaver Bell Societe Anonyme v. North Lake Marketing and Supply Inc.* 45 F.3d 1550 33 USPQ 2d 1496 (Fed. Cir. 1995). In other words, as stated in MPEP § 706.02, for anticipation under 35 U.S.C. §102, the reference must teach every aspect of the claimed invention either explicitly or impliedly.

Since the reference cited does not teach 1) separation/retention step for a low molecular weight protein fraction that includes proteins above about 3 kDa for analysis and 2) uses different process steps, Myrick et al. does not meet every limitation of the claimed invention. Therefore, Myrick et al. does not anticipate claims 1 and 3, 8, 9 and 17 (amended in part). Moreover, the rejection does not read on new claims 25-37 since said claims are

dependent from claim 1 ("dependent claims of [an independent] claim . . . are to construed to incorporate by reference all the limitations of [the independent] claim . . . ." *Robotic Vision Systems, Inc., v. View Engineering, Inc.*, 189 F.3d 1370, 1376, 51 USPQ2d 1948, 1953 (Fed. Cir. 1999)).

Respectfully, in view of the arguments presented above, Applicants submit that the rejection, as it might be employed against the instant amended or new claims, does not apply.

VIII. Rejections Under 35 U.S.C. §103(a)

A. Claims 1-5, 8-11, 17 and 19 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Spahr et al. (Proteomics (2001) 1:93-97) in view of Liu et al (U.S. Pat. No. 5,492,834). Applicants respectfully traverse the rejection as it stands against claims 1-5, 8-11, 17 and 19 (amended in part).

To establish a *prima facie* case of obviousness, three basic criteria must be met. First there must be some suggestion or motivation in the references then or in knowledge generally available to one of skill in the art, to modify the reference or combine the reference teachings. Second, there must be a reasonable expectation of success. And, finally the prior art reference (or references when combined) must teach all claim limitations. The teaching or suggestion and reasonable expectation of success must both be found in the prior art and not in Applicants' disclosure. (see MPEP § 706.02(j)).

The Examiner asserts that Spahr et al. disclose a method to detect proteins in urine samples using centrifugation, fractionation, electrophoresis and LC-MS/MS. Further, the

Examiner states that the reference fails to disclose separation of low molecular weight by size exclusion chromatography.

However, closer examination of the Spahr et al. demonstrates that the first step requires that the urine sample be denatured, reduced and enzymatically digested prior LC-MS/MS (for 2DE, urinary proteins reduced and heated prior to loading, there is no prior chromatography). The resulting complex of products of such a process would not be a mixture of low molecular weight products as in the claimed invention. Moreover, for either LC-MS/MS or electrophoresis there is no teaching or suggestion of using non-affinity chromatography for isolation and analysis of a fraction having proteins or peptides with a molecular weight above about 3 kDa and below the filtration limits of a normal kidney prior to the first denaturing step (e.g., prior to electrophoresis).

Liu et al. teach that the only pre-treatment of the sample prior to analysis involves the use of molecular weight exclusion (i.e., no denaturation/reduction/digestion). The primary reference neither teaches nor suggests that the initial denaturing/reducing/digesting of the samples be modified to remove this step nor does the secondary reference teach or suggest that the pretreatment prior to analysis include denaturing/reducing/digesting of samples prior to gel exclusion. Thus, there is no motivation or suggestion to substitute the first step of Spahr et al. for the first step of Liu et al (or vice versa).

Further, the suggestion by Spahr et al. to use affinity chromatography would defeat the purpose of Liu et al. since the very same proteins to be removed in the former are the analyte targets of the latter (e.g., albumin, transferrin, $\alpha 2$ and immunoglobulin). Therefore,

one of skill in the art would have no reasonable expectation of success. Moreover, the combination fails to teach all of the claims limitations (e.g., separating a fraction having proteins or peptides with a molecular weight above about 3 kDa and below the filtration limits of a normal kidney).

Applicants submit that the Examiner has failed to meet the burden of establishing a *prima facie* case of obviousness for the reasons recited above.

B. Claim 7 stands rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Spahr et al. (Proteomics (2001) 1:93-97) in view of Liu et al. (U.S. Pat. No. 5,492,834) as applied to claims 1-5, 8-11, 17 and 19 and in further view of O'Donnell et al (U.S. Pat. No. 5.998,216). Applicants respectfully traverse the rejection as it stands against claims 1-5, 7-11, 17 and 19 (amended in part).

The Examiner alleges that O'Donnell et al. discloses the use of protease inhibitors in urine to preserve the samples, more specifically to effect stability of cytokines. However, Applicants point out that the addition of protease inhibitors would be counter-intuitive since the goal of the Spahr et al. reference would be to digest the protein in the urine sample as the first step. If the primary reference teaches the skilled artisan to add N-tosyl-Lys-chloromethyl-ketone-trypsin (TPCK) to the urine sample (i.e., at page 94, column 1, paragraph 2.1) and O'Donnell et al. teaches one of skill the addition of protease inhibitors to the urine sample, then it would seem counter-intuitive to combine these teachings when one reference defeats the purpose of the other (i.e., TPCK would be inhibited by soybean trypsin inhibitor). Therefore, one of skill in the art would have no reasonable expectation of success.

Applicants submit that the Examiner has failed to meet the burden of establishing a *prima facie* case of obviousness for the reasons recited above.

C. Claims 12-14 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Spahr et al. (Proteomics (2001) 1:93-97) in view of Liu et al. (U.S. Pat. No. 5,492,834) as applied to claims 1-5, 8-11, 17 and 19 and in further view of Suzuki et al (U.S. Pat. No. 5,246,835). Applicants respectfully traverse the rejection as it stands against claims 1-5, 8-11, 12-14, 17 and 19 (amended in part).

The Examiner alleges that Suzuki et al. disclose an affinity column coupled with monoclonal or polyclonal antibody specific for albumin. Applicants submit that Suzuki et al. do not cure the deficiencies identified, *supra*, in the Spahr et al. and Liu et al. references. Further, Suzuki et al. does not teach or suggest modification of the prior two references to achieve the invention as claimed.

Applicants submit that the Examiner has failed to meet the burden of establishing a *prima facie* case of obviousness for the reasons recited above.

D. Claims 15 and 16 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Spahr et al. (Proteomics (2001) 1:93-97) in view of Liu et al. (U.S. Pat. No. 5,492,834) and Suzuki et al. (U.S. Pat. No. 5,246,835) as applied to claims 1-5, 8-14, 17 and 19 and in further view of Degen et al (U.S. Pat. No. 4,693,985).

Applicants respectfully traverse the rejection as it stands against claims 1-5, 8-17 and 19 (amended in part).

The Examiner alleges that Degen et al. disclose an affinity column coupled with protein A to remove mammalian proteins from body fluids. As with Suzuki et al., Degen et al. do not cure the deficiencies identified, *supra*, in the Spahr et al. and Liu et al. [or Suzuki et al.] references. Further, Degen et al. do not teach or suggest modification of the prior three references to achieve the invention as claimed. Applicants submit that the Examiner has failed to meet the burden of establishing a *prima facie* case of obviousness for the reasons recited above.

E. Claims 6 and 18 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Spahr et al. (Proteomics (2001) 1:93-97) in view of Liu et al. (U.S. Pat. No. 5,492,834) as applied to claims 1-5, 8-11, 17 and 19 and in further view of Furst et al (U.S. Pat. No. 5,926,387).

Applicants respectfully traverse the rejection as it stands against claims 1-6, 8-11 and 17-19 (amended in part).

The Examiner alleges that Furst et al. disclose the use of zonal sedimentation, which is suggested to improve the efficiency of fractionation by separating the particles according to size. As with Suzuki et al. and/or Degen et al., Furst et al. do not cure the deficiencies identified, *supra*, in the Spahr et al. and Liu et al. references. Further, Furst et al. do not teach or suggest modification of the prior two references to achieve the invention as claimed.

Applicants submit that the Examiner has failed to meet the burden of establishing a *prima facie* case of obviousness for the reasons recited above.

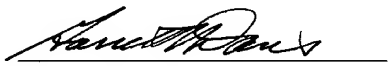
F. Claims 20-24 stand rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Spahr et al. (Proteomics (2001) 1:93-97) in view of Liu et al. (U.S. Pat. No. 5,492,834) as applied to claims 1-5, 8-11, 17 and 19 and in further view of Taylor, Jr. et al (U.S. Pat. No. 6,301,377). Applicants respectfully traverse the rejection as it stands against claims 1-5, 8-11, 17 and 19-24 (amended in part).

The Examiner alleges that Taylor et al. disclose a method of warping a plurality of gel electrophoresis images, including bringing multiple gel images into register for comparison. While not acquiescing to the reasoning offered by the Examiner, and to expedite prosecution toward allowance, Applicants have canceled claims 20-24 to obviate this rejection.

CONCLUSION

Applicants have taken substantial steps to advance prosecution. Reexamination, reconsideration, withdrawal of the rejections and allowance are requested.

Respectfully submitted,



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MARKED UP CLAIMS
09/921,004

1. (Amended) A method of detecting at least one low molecular weight protein and/or peptide component in a biological fluid comprising

(a) fractionating proteins or peptides in said biological fluid by molecular weight to produce a fractionated protein or peptide sample;

(b) separating a first fraction from said fractionated proteined or peptide sample, said first fraction having proteins or peptides with a molecular weight above about 3 kDa and below the filtration limits of a normal kidney; [and]

(c) recovering [each] said first fraction having proteins or peptides with a molecular weight above about 3kDa and below the filtration limits of a normal kidney, and

(d) determining the proteins or peptides present in said first fraction.

5. (Amended) The method of claim 1, wherein said fractionating [concentrating] step comprises separation of low molecular weight constituents by size exclusion chromatography.

8. (Amended) The method of claim 1, wherein said [concentrating step] fractionating comprises a hydrodynamic step.

10. (Amended) The method of claim 1, [wherein said] further comprising fractionating said first fraction by [step comprising the] elution from a reverse phase stationary phase.

12. (Amended) The method of claim 1, wherein said first fraction is further fractionated [fractionating step further comprises] by elution from an affinity column.

13. (Amended) The method of claim 12, wherein said affinity column comprises monoclonal, polyclonal, [or] recombinant, microorganism display antibodies, or fragments thereof.

14. (Amended) The method of claim 13 [14], wherein said [monoclonal and/or polyclonal] antibodies are directed to target proteins selected from the group consisting of albumin, transferrin, α_1 antitrypsin, [and] $\alpha_2$macroglobulin, $\alpha_1$acid glycoprotein, C3, Tamm-Horsfall protein, hemopexin, α_2HS glycoprotein, $\alpha_1$antichymotrypsin, Gc globulin and ceruloplasmin.

15. (Amended) The method of claim 12 [13], wherein said affinity column [chromatography] is a non-immunologic entity comprising matrix.

16. (Amended) The method of claim 15 [16], wherein said non-immunologic entity is selected from the group consisting of protein A, protein G, haptoglobin, arginine, benzamidine, glutathione, Cibacron blue, calmodulin, gelatin, heparin, lysine, lectins, Procion Red HE-3B, nucleic acids and metal affinity media.

17. (Amended) The method of claim 1, wherein said first fraction is further fractionated by electrophoresis [separating steps comprises two-dimensional electrophoresis (2DE)].

18. (Amended) The method of claim 1, wherein said [separating step comprises] first fraction is further fractionated by zonal sedimentation centrifugation on density gradients.

19. (Amended) The method of claim 1, wherein said determining step [deflecting step] comprises [time of flight] identifying said proteins or peptides by mass spectrometry or liquid chromatography.

constituted so to select predetermined graphic data from among the graphic data stored in the graphic storing means based on coordinate data specified by a cursor means displayed and moveable on the display means.

In a further aspect, the instant invention relates to the analysis of low molecular weight proteins in body fluids, such as urine, which low molecular weight proteins can be used as an indicator of tissue damage.

These and other advantages associated with the present invention and a more detailed explanation of preferred embodiments are described below and should be taken in combination with the following drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

~~Figure 1 depicts a map of separated human plasma proteins.~~

Figure 1A depicts acidic high molecular weight plasma proteins.

Figure 1B depicts basic high molecular weight plasma proteins.

Figure 1C depicts acidic low molecular weight plasma proteins.

Figure 1D depicts basic low molecular weight plasma proteins.

Figures 2A-2C depict gel filtration scans of plasma and urine. Figure 2C provides molecular weight standards.

Figure 3 is a histogram of urinary proteins.

DETAILED DESCRIPTION OF THE INVENTION

As used herein the term "deflecting", including grammatical variations thereof refers to turn aside especially from a straight course or fixed direction.

As used herein the term "cognizable", including grammatical variations thereof refers to as capable of being known.

As used herein the term "body fluid", including grammatical variations thereof refers to liquid components of living organisms. For example, blood, lymph,